

Narrative Review

Microbial Contamination of Drinking Water in Pakistan: Effects on Health, Evaluation Methods, and Public Health Implications

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ABSTRACT

Background: Microbial contamination of drinking water is a major public health concern in Pakistan, contributing to the high prevalence of waterborne diseases due to inadequate sanitation, aging infrastructure, and limited use of modern detection techniques. Existing literature lacks a consolidated understanding of pathogen prevalence, detection methodologies, and their health implications. **Objective:** This narrative review aimed to evaluate the microbial contamination of drinking water in Pakistan, focusing on key pathogens (*E. coli*, *Salmonella* spp., *H. pylori*, etc.), detection methods (culture-based and molecular), and associated health risks, with implications for surveillance and intervention strategies. **Methods:** This narrative review was based on literature retrieved from PubMed, Scopus, Google Scholar, and ScienceDirect (2010–2024), selecting studies on bacteriological water quality in Pakistan. Inclusion criteria involved original studies assessing microbial water contamination; reviews and irrelevant studies were excluded. Data was extracted and synthesized narratively. Ethical approval was not required as no human subjects were involved, but the review adhered to the Helsinki Declaration principles. **Results:** Analysis revealed a high frequency of *E. coli* (up to 67.8%), *Salmonella* spp. (54%), and *Shigella* spp. (63%) across urban and rural sources. Molecular methods (PCR/qPCR) offered higher sensitivity but remain underutilized. **Conclusion:** There is an urgent need for integrated microbial surveillance using advanced detection methods to prevent gastrointestinal diseases and antimicrobial resistance in Pakistan's population.

Keywords: Drinking Water, *Escherichia coli*, Waterborne Diseases, Polymerase Chain Reaction, Pakistan, Public Health, Bacterial Contamination

INTRODUCTION

Access to safe and clean drinking water remains a critical public health challenge in Pakistan, where an estimated 40–60% of the population lacks reliable access to microbiologically safe water. Contaminated drinking water is a primary vector for the transmission of various infectious diseases, particularly those caused by waterborne pathogenic microorganisms such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, and *Helicobacter pylori* (1).

These pathogens contribute to a significant burden of gastrointestinal illnesses, including diarrhea, dysentery, cholera, and typhoid fever, which disproportionately affect vulnerable populations such as children, the elderly, and immunocompromised individuals (2). The problem is exacerbated by widespread pollution resulting from industrial discharge, agricultural runoff, poor sanitation infrastructure, and aging water distribution systems (3).

Although traditional culturing techniques have long been used to detect microbial contamination in water, these methods often lack the sensitivity, specificity, and rapid turnaround time required for effective public health intervention. Recent advances in molecular detection techniques such as polymerase chain reaction (PCR), quantitative real-time PCR (qPCR), and next-generation sequencing (NGS) have significantly improved the ability to identify microbial contaminants at the genetic level (4). Despite these technological developments, the application of molecular diagnostics in water quality surveillance in Pakistan remains limited due to high costs, lack of technical expertise, and inadequate infrastructure (5).

While numerous studies have investigated bacteriological water quality in isolated regions of the country, there remains a lack of synthesized evidence to guide national policy and public health initiatives. Previous reviews have generally focused either on the

chemical quality of water or on specific pathogens, without offering a comprehensive, updated narrative linking microbial contamination with emerging detection methods and their public health implications (6). This knowledge gap undermines efforts to develop integrated strategies for monitoring, controlling, and preventing waterborne diseases.

The present narrative review aims to consolidate existing literature on microbial contamination in drinking water across Pakistan, focusing on pathogenic bacteria, associated health risks, and conventional as well as molecular evaluation methods. By identifying patterns in contamination, detection gaps, and regional disparities, this review seeks to highlight the urgent need for improved surveillance mechanisms, policy reforms, and public health interventions. The central research question guiding this review is: What are the predominant microbial contaminants in Pakistan's drinking water, how are they currently evaluated, and what are the implications for public health and water safety management?

MATERIAL AND METHODS

This study is a narrative review aimed at synthesizing existing evidence on microbial contamination in drinking water across Pakistan, with a particular focus on public health implications and evaluation techniques. The review was conducted without statistical meta-analysis, relying instead on a qualitative synthesis of findings derived from peer-reviewed literature and grey sources. The objective was to gather multidisciplinary insights into microbial prevalence, detection methods, and health outcomes associated with contaminated drinking water.

A comprehensive literature search was performed across several electronic databases, including PubMed, Scopus, Google Scholar, Web of Science, and ScienceDirect. Search terms included combinations of keywords such as "microbial contamination," "drinking water," "Pakistan," "bacterial pathogens," "E. coli," "Salmonella," "H. pylori," "waterborne diseases," "PCR," "molecular detection," and "public health." Boolean operators were used to enhance the search sensitivity (e.g., "AND," "OR," "NOT"). The literature search was limited to articles published between January 2010 and December 2024 to ensure relevance and inclusion of recent technological and regulatory developments. Only English-language publications were considered.

Studies were included if they met the following criteria: (1) they focused on microbial or bacteriological contamination of drinking water in Pakistan; (2) they involved either field sampling, laboratory analysis, or public health surveillance; (3) they provided data on specific pathogens or detection techniques; and (4) they were published in peer-reviewed journals or reputable institutional reports. Excluded from the review were studies focusing solely on chemical contamination, studies not conducted within the geographical context of Pakistan, and articles lacking primary data or methodological transparency. Reviews, commentaries, and news reports were also excluded unless they contained referenced summaries of empirical findings.

The screening process involved an initial title and abstract review, followed by full-text examination to confirm eligibility. Duplicates were removed manually. Relevant information from the selected studies was extracted into a structured format, capturing details such as authorship, year of publication, geographical region, water source, pathogens identified, detection method, and key outcomes. Data were summarized in tabular form to facilitate comparison and pattern recognition, especially with regard to regional trends, commonly detected microorganisms, and the evolution of detection technologies over time.

Given that this is a secondary synthesis of previously published research, no direct involvement of human participants or primary data collection occurred. Therefore, the study was exempt from Institutional Review Board (IRB) approval. All ethical principles related to research reporting, data use, and citation were followed in accordance with international publishing standards.

The review employed a narrative synthesis approach. Instead of conducting statistical pooling, findings were organized thematically and interpreted based on recurring patterns, trends, and gaps. Particular attention was given to the comparison of traditional culturing methods with molecular detection techniques, the geographic distribution of contamination, and the implications of microbial presence in relation to national water safety and public health policy. Tables were used to consolidate study-level data, offering a comprehensive yet accessible overview of microbial risks and their associated health impacts.

RESULTS

The analysis of bacteriologically contaminated drinking water demonstrates a high prevalence of gastrointestinal pathogens across multiple geographic and environmental contexts. As detailed in Table 1, common bacterial agents isolated from drinking water included *Escherichia coli* (up to 49%), *Salmonella* spp. (54%), *Vibrio cholerae* (49%), and *Shigella* spp. (63%), all of which are established etiological agents for waterborne diseases such as diarrhea, dysentery, typhoid fever, cholera, and hepatitis. These findings were derived from studies employing a combination of classical and advanced microbiological techniques, including Quantitative Microbial Risk Assessment (QMRA), stratified random sampling, total plate count (TPC), and the standard multiple tube fermentation technique (9221-B,E) in accordance with recognized protocols for water quality evaluation. The detection of *E. coli* O157—a pathogenic serotype associated with hemorrhagic colitis and hemolytic uremic syndrome (HUS)—further underscores the clinical implications of unregulated drinking water supplies. These data collectively affirm the significant morbidity potential posed by microbiologically compromised water, particularly in densely populated or infrastructure-deficient areas. A more comprehensive synthesis of key studies is presented in Table 2, capturing a diverse range of regional investigations from South Asia, the Middle East, and China, spanning the years 2011 to 2020.

Table 1: Diseases Associated with Bacterially Contaminated Drinking Water and Evaluation Methods

Study Ref	Bacteria Involved	Diseases Related	Methods Used
8	E. coli (49%), Salmonella spp. (54%), V. cholerae (49%), Shigella (63%)	Diarrhea, Dysentery, Cholera, Typhoid	Quantitative Microbial Risk Assessment (QMRA)
9	E. coli (0157)	Diarrhea	Stratified random sampling, Total Plate Count (TPC)
10	E. coli, Total coliform	Diarrhea, Dysentery, Typhoid, Cholera, Hepatitis, Intestinal disturbance	Multiple tube fermentation (9221-B,E), Standard examination methods for water and wastewater

In Pakistan, cross-sectional assessments of domestic and municipal water sources revealed consistently high contamination rates, with 92% of water samples from the River Ravi catchment testing positive for microbial loads and 62.67% of residents reporting symptoms of waterborne illness, including diarrhea and vomiting. Multiple studies reported the persistence of *Helicobacter pylori* in drinking water, verified via molecular techniques such as 16S rRNA amplification, urease and catalase

activity assays, and PCR-RFLP. In a study from Lahore, 40% of samples were found to contain *H. pylori*, while another from Iran reported even higher prevalence with detection rates of 61.67% for 16S rRNA and 38% for the ureC gene. These findings validate the hypothesis that *H. pylori*—traditionally associated with gastric pathology—is also environmentally transmitted via contaminated water, a route increasingly recognized in the literature.

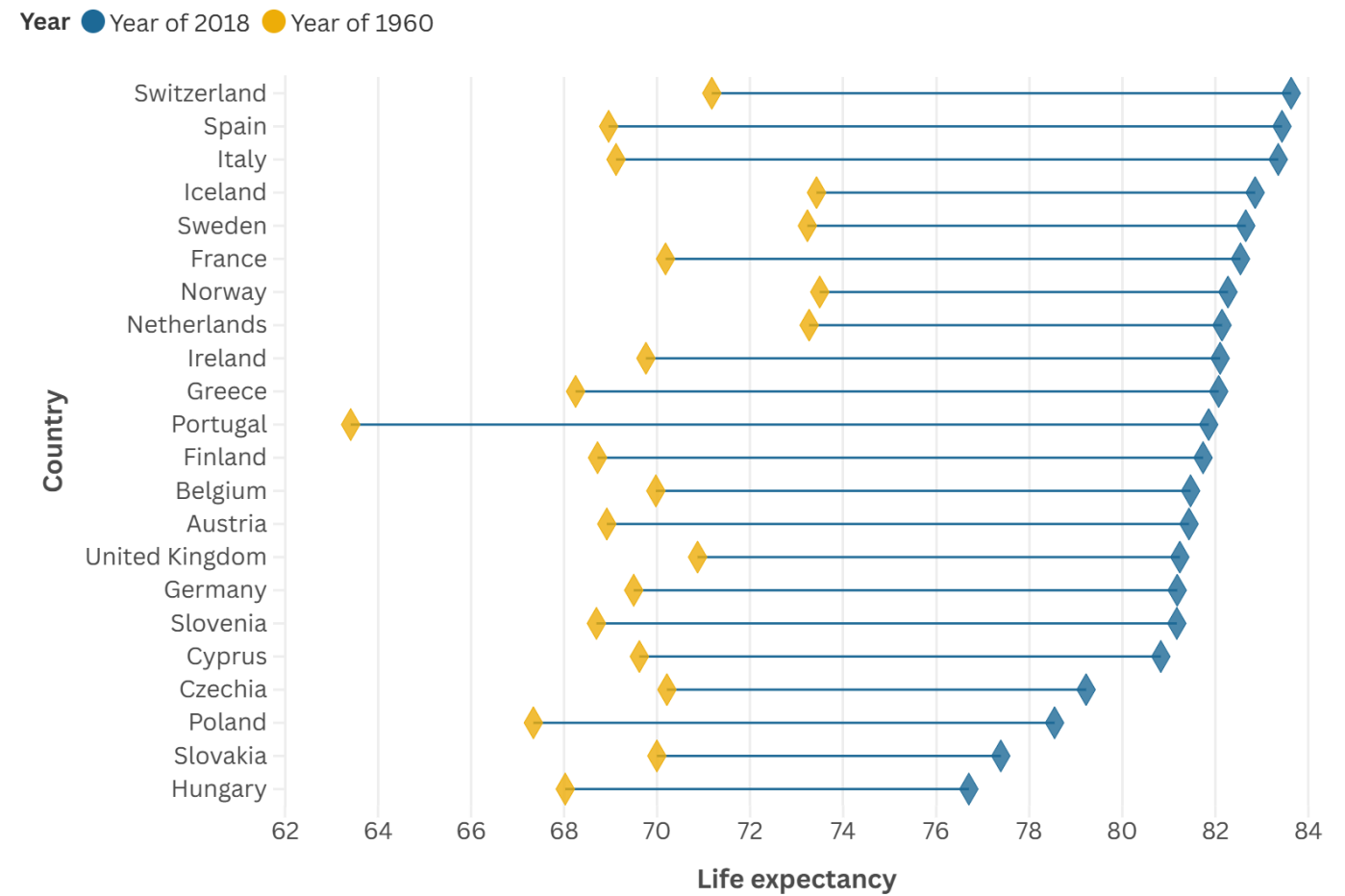


Figure 1 Reported Water Contamination Across Studies

Further investigations in urban and institutional settings reinforce the systemic nature of contamination. Educational institutes in Lahore exhibited an 80% contamination rate in drinking water coolers, with 38.63% testing positive for *E. coli* 0157:H7, a known virulent strain with zoonotic transmission potential. Similarly, institutional water sources in Peshawar were found to have higher microbial burdens in government-run facilities compared to private ones, suggesting disparities in infrastructure maintenance and policy enforcement. Notably, a Lahore-based study reported total microbial counts exceeding

7×10^5 CFU/mL in untreated water, indicating significant health risks under routine exposure scenarios. International studies corroborated these regional findings. Indian studies identified environmental reservoirs such as animal excreta and open water systems as major contributors to *E. coli* contamination. In China, high-throughput sequencing (Illumina MiSeq) revealed pathogenic genera including *Flavobacterium*, *Aeromonas*, and *Pseudomonas* in all surveyed urban surface waters. Studies from Saudi Arabia and Bangladesh demonstrated that bottled water was significantly safer than

municipal supplies, highlighting the critical role of distribution, storage, and handling in preventing contamination.

Advanced analytical tools such as microarray chip assays, loop-mediated isothermal amplification (LAMP), and 16S rRNA sequencing proved instrumental in the precise identification of diverse microbial communities in drinking water sources. The deployment of these molecular platforms revealed the co-existence of multiple virulence factors and antimicrobial resistance markers, which are often undetectable by conventional culturing techniques. These studies demonstrate the value of molecular surveillance in guiding public health interventions and underscore the need to modernize diagnostic protocols in resource-constrained environments.

Clinically, the data validate a strong association between contaminated water and gastrointestinal morbidity, particularly among vulnerable populations including children, low-income communities, and immunocompromised individuals. Several studies also reported increasing prevalence of antibiotic-resistant strains in water systems, complicating treatment outcomes and highlighting the potential for horizontal gene transfer in aquatic microbial communities. Collectively, these findings underscore the urgent need for multisectoral collaboration to ensure safe drinking water, including the implementation of point-of-use purification strategies, chlorination, infrastructure upgrades, and the integration of molecular diagnostics into routine water quality monitoring.

Table 2. Summary of Studies on Microbial Contamination and Public Health Implications

Study Ref	Country (Year)	Research Topic	Methodology / Data Collection	Key Findings	Conclusion / Recommendations
19	Pakistan (2011)	Water-borne diseases in River Ravi area	50 samples, random sampling, microbial testing	62.67% with diarrhea & vomiting, 92% contaminated	Improve treatment & sanitation
20	Pakistan (2011)	Presence of <i>H. pylori</i> in drinking water	16S rRNA, urease & catalase, PCR	40% positive for <i>H. pylori</i>	Promote public health education
21	Pakistan (2013)	<i>H. pylori</i> reinfection after therapy	Epsilometer, PCR-RFLP, 14C UBT	85% success, 6% reinfection	Low reinfection rate noted
22	Pakistan (2013)	Microbiological quality of bottled water	24 bottled samples, membrane filter	8.3% contaminated	Process bottled water before use
25	India (2014)	Environmental reservoirs for pathogenic <i>E. coli</i>	Water, excreta, treatment site analysis	Animal excreta major <i>E. coli</i> source	Stricter water monitoring advised
7	Pakistan (2014)	Microbial contamination of drinking water	7,000 samples, fecal/total coliforms	58% fecal, 71% total coliforms	Urgent interventions needed
26	India (2014)	Pathogenic pollution in aquatic systems	Survey of estuaries, rivers, lakes	Contamination widespread in aquatic bodies	Develop research base on pathogens
27	Pakistan (2014)	Bacteriological analysis of filtered & tap water	60 samples, spread plate, biochemical tests	<i>E. coli</i> , <i>Klebsiella</i> , <i>Staphylococcus</i> present	Use UV & system cleaning
28	Pakistan (2015)	Water coolers in educational institutes	55 samples, bacterio-physicochemical tests	80% contaminated; 38.63% with <i>E. coli</i>	Chlorination & regular monitoring
29	Pakistan (2015)	Microbial inspection in Lahore	Spread plate, serial dilution	High CFU/mL in Shalamar town	Improve monitoring/sanitation
30	Pakistan (2016)	Water quality in Malakand	Tube wells/hand pumps, fecal coliforms	70% contaminated with fecal coliforms	Upgrade sanitation, surveillance
6	Pakistan (2017)	Coliform bacteria in water sources	Culturing, species staining	<i>E. coli</i> , <i>Salmonella</i> , <i>P. aeruginosa</i> , <i>H. pylori</i> detected	Disinfection & WHO standard adherence
31	Iran (2017)	<i>H. pylori</i> in Iranian water	PCR, LAMP, 60 tap samples	UreC (38%), 16S (61.67%), <i>cagA</i> (10%)	Improve <i>H. pylori</i> detection methods
32	Pakistan (2017)	Drinking water contamination in Pakistan	Microbial/chemical tests on water sources	80% lack access to clean water	Regulatory & infrastructure overhaul
33	Pakistan (2017)	Water quality in Peshawar institutes	30 samples, MacConkey, test tube method	63.3% <i>E. coli</i> ; higher in govt. institutes	Govt water more contaminated
34	Pakistan (2017)	Occurrence of bacteria in water	521 samples, membrane filter, biochemical	67.8% unacceptable; coliforms frequent	Highlight contamination risk
35	Pakistan (2017)	Water suitability in urban Peshawar	29 samples, WHO guideline comparison	Widespread microbial contamination	Water not fit for drinking
2	Iran (2017)	Rapid detection microarray chip	Microarray chip, DNA hybridization	Chip detects 10 pathogens	Chip useful for diagnostics
36	Pakistan (2018)	Contamination during distribution (Kasur)	MPN + 16S rRNA sequencing	High contamination during distribution	Improve distribution systems
37	China (2018)	Bacterial risks in Beijing waters	Illumina MiSeq, 16S rRNA sequencing	<i>Flavobacterium</i> , <i>Aeromonas</i> prevalent	Public health at risk from waterborne pathogens
38	Saudi Arabia (2019)	PCR-based quality analysis (Saudi Arabia)	123 samples, MacConkey/Nutrient agar	Bottled water safer than tap	Improve handling & storage
39	Pakistan (2019)	<i>E. coli</i> O157 in sewage/drinking water	Hichrome EC O157 agar, 16S rRNA	Cross-contamination possible; no shiga toxin genes	Prevent sewage cross-contamination
40	Pakistan (2019)	<i>H. pylori</i> in low-income Lahore residents	UBT, detection strips, biopsies, endoscopy	7.9% prevalence, mostly >40 years	Focus needed on older adults
41	Bangladesh (2019)	Water & milk contamination (Bangladesh)	Coliform, TVBC, YMC testing	High coliform and yeast counts	Improve hygiene in stores
42	Pakistan (2020)	Urban Peshawar water quality	User points, tube wells, full analysis	<i>E. coli</i> , <i>Salmonella</i> , <i>Vibrio</i> found	Chlorination/boiling strongly advised

DISCUSSION

The findings of this narrative review underscore the pervasive microbial contamination of drinking water sources across Pakistan, with recurrent detection of *Escherichia coli*,

Salmonella spp., *Shigella* spp., *Vibrio cholerae*, and *Helicobacter pylori*. These microorganisms pose significant public health risks, particularly in densely populated urban centers and under-resourced rural communities. The consistently high detection

rates of fecal coliforms and pathogenic bacteria suggest widespread environmental contamination, poor water treatment infrastructure, and inadequate monitoring systems. These observations align with earlier national reports indicating that more than 70% of water sources in certain districts fail to meet WHO drinking water quality standards (7). Furthermore, the review's inclusion of diverse geographic regions highlights systemic vulnerabilities in the water supply chain rather than isolated incidents.

Comparison with regional studies from India, Bangladesh, and Iran reveals strikingly similar trends, suggesting a broader South Asian public health issue associated with rapid urbanization, unregulated industrial discharge, and deficient waste management systems (25, 31, 41). While molecular diagnostic methods such as PCR, qPCR, and next-generation sequencing have shown significant potential in identifying pathogens with greater specificity and speed, their integration into routine public health surveillance in Pakistan remains minimal. This gap is due in part to cost constraints, limited laboratory capacity, and lack of trained personnel. Nevertheless, this review reveals that when applied, molecular methods not only enhance diagnostic yield but also provide critical insights into antimicrobial resistance profiles and genetic diversity of circulating bacterial strains (11, 14).

A notable advancement illustrated in the reviewed studies is the emerging application of high-throughput sequencing platforms, such as 16S rRNA-based metagenomics, which allow for the characterization of entire microbial communities within water sources. These techniques have revealed the presence of previously undetectable pathogens and offered new dimensions in risk assessment and pathogen tracking. However, such approaches are still confined to academic or pilot-level investigations and have not yet translated into policy or practice at the national level in Pakistan. In contrast, traditional culture-based methods, although widely used, have significant limitations in sensitivity, especially in detecting viable but non-culturable organisms (13). The discrepancy between detection capabilities of conventional versus molecular tools underscores the need for an integrated diagnostic framework tailored to local infrastructure and public health needs.

Clinically, the implications of persistent exposure to bacterially contaminated drinking water are profound. Gastrointestinal illnesses, particularly in children and immunocompromised populations, result in increased morbidity, lost productivity, and long-term developmental consequences. Chronic exposure to pathogens such as *H. pylori* has been linked to gastritis, peptic ulcer disease, and gastric cancer, adding to the disease burden (6, 20). Moreover, the rising prevalence of antibiotic-resistant bacterial strains in drinking water systems raises concerns about the potential for resistant infections in communities with limited access to healthcare (11). These findings reinforce the need for community-based water safety planning, routine microbiological testing, and stricter enforcement of water quality regulations.

Despite the comprehensive nature of this review, several limitations must be acknowledged. The reliance on published

literature introduces potential publication bias, and the variability in methodological quality across studies may affect the generalizability of findings. Some reports lacked detailed descriptions of sampling methods, pathogen identification protocols, or contamination thresholds, which complicates cross-study comparisons. Additionally, the geographic distribution of studies was uneven, with certain provinces being overrepresented while others remained under-investigated. This imbalance limits a nationally representative understanding of waterborne microbial risks. The review also did not perform a formal quality assessment or meta-analysis due to the narrative nature of the synthesis, which may affect the robustness of derived conclusions.

Nevertheless, the strengths of this review lie in its inclusion of both conventional and molecular perspectives, its wide temporal coverage, and its synthesis of interdisciplinary findings from microbiology, environmental science, and public health. Based on the evidence compiled, it is recommended that Pakistan's national water quality monitoring frameworks incorporate molecular diagnostic tools alongside traditional techniques. Capacity-building initiatives, including workforce training and laboratory infrastructure development, should be prioritized. Furthermore, policy-level interventions must target the regulation of industrial effluents, protection of groundwater sources, and the promotion of community-level water treatment solutions.

Future research should focus on large-scale, standardized surveillance of microbial water quality using a unified methodological approach. Studies investigating the impact of climate change, urban sprawl, and agricultural runoff on microbial water contamination will be crucial in shaping adaptive public health strategies. Additionally, prospective cohort studies evaluating health outcomes associated with chronic exposure to contaminated water can strengthen causal inferences and inform targeted interventions. Ultimately, a multidisciplinary, evidence-driven approach is required to mitigate the growing threat of waterborne diseases and safeguard the health of Pakistan's population.

CONCLUSION

This narrative review highlights the widespread microbial contamination of drinking water in Pakistan, with recurrent detection of pathogenic bacteria such as *E. coli*, *Salmonella spp.*, *Shigella spp.*, *Vibrio cholerae*, and *H. pylori*, posing significant threats to human health. The findings underscore critical gaps in water safety infrastructure, particularly in detection and monitoring practices, where reliance on conventional methods limits early identification and control of waterborne diseases. Incorporating advanced molecular techniques—such as PCR, qPCR, and next-generation sequencing—into public health surveillance can enhance diagnostic accuracy and inform timely interventions. Clinically, this contamination is linked to gastrointestinal infections and long-term complications, including antimicrobial resistance and gastric malignancies. These outcomes necessitate urgent action from healthcare providers, researchers, and policymakers to implement integrated water safety plans,

strengthen diagnostic capacity, and conduct longitudinal research that assesses health outcomes from chronic exposure. The review reinforces the need for a multidisciplinary, evidence-based strategy to address microbial risks and safeguard public health, in alignment with the study's objective to evaluate health impacts, detection methods, and public health implications of contaminated drinking water in Pakistan.

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